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CHROMOSOME STRUCTURAL CHANGES IN TRADESCANTIA MICROSPORES PRODUCED BY ABSORBED RADIOPHOSPHORUS

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The utilization of radioisotopes in biological experimentation up to the present time has been mainly concerned with their effectiveness as tracers. There is also the possibility of using the radiations from such substances in producing various cytogenetical changes. Since radioactive isotopes of many elements normally occurring in protoplasm are now available[1] and can be introduced directly into the cells and tissues of organisms, it seemed of particular interest to compare any resulting cytogenetical effects with those produced by various external sources of radiations, about which a considerable amount of information has been accumulated.[2] The present paper deals with preliminary observations of the effects of radiophosphorus absorbed by inflorescences of *Tradescantia* in producing chromosomal structural changes in microspore nuclei.

Experimental Methods.—The radiophosphorus used (obtained from the Clinton Laboratories at Oak Ridge) was the separated isotope, phosphorus 32; half-life: 14.3 days, β-radiation: 1.69 Mev. The original solution, whose concentration (activity) when obtained was approximately 600 microcuries (μc)./ml., was used in four dilutions: A—100 μc./ml., B—10 μc./ml., C—1 μc./ml., D—0.1 μc./ml. A clone of *Tradescantia paludosa* was utilized for all cytological observations. The cut ends of ten inflorescences were inserted through holes in lead shields placed over each of four small beakers containing approximately 40 ml. of the dilutions of radiophosphorus used. The lead shields made certain that any effects would be due to the radiophosphorus actually taken up by the plants and not to radiation from the solution. In all the experiments, unless otherwise noted, the inflorescences remained in the solution throughout the period of observation. Further lead shielding was utilized about the inflorescences as protection against emitted β-radiation.

Measurements of the increase in radioactivity due to the uptake of

phosphorus were made with a separate group of inflorescences, utilizing a Lauritsen electroscope. There was a significant increase in activity over the background within about an hour and an increasing rate of activity continued until approximately eight hours after the initiation of the experiment. At this time the rate of increase in activity became approximately constant and remained so for at least four days. Numerous measurements were also made of the activity resulting from phosphorus uptake by individual buds or anthers with a Geiger-Müller counter. In some instances such buds were immediately examined cytologically, making possible a direct comparison of the amount of radioactivity and the aberration frequency.

Aceto-carmine smear preparations of microspores were made at intervals following the start of the experiments and analyzed for chromosome structural changes at the first post-meiotic mitosis.

Results.—The results of cytological analyses at three concentrations of radiophosphorus are presented in table 1. With the highest concentration (A—100 μc./ml.) satisfactory observations were possible at 24 hours after the treatment started, but the aberration frequency soon became too extensive to permit complete analysis. In a few instances where inflorescences were placed in this concentration of radiophosphorus for a limited period of time and then removed, aberration frequencies were much lower and complete analyses were possible. The types of chromosome structural change found are similar to those induced by other radiations such as x-rays and neutrons.[3, 4, 5] In experiments utilizing external radiations, inflorescences are usually exposed for a brief period to the radiation and cytological examination then made at intervals following treatment. It is found that chromatid aberration types appear at metaphase within a few hours, having been induced in cells in prophase with divided chromosomes at the time of treatment. There is then a transition period with a mixture of types present at about 30 to 40 hours following irradiation after which only chromosome aberration types, produced in cells in the resting stage with effectively unsplit chromosomes, are found. With radiophosphorus treatment chromatid types are also observed initially, and may be quite frequent within 24 hours at higher concentrations—at 100 μc./ml. (series A) the frequency of chromatid and isochromatid breaks per 100 cells was 35. Furthermore, table 1 indicates that these types continue to represent the principal component of the aberrations observed up to nine days after the start of treatment with initial phosphorus concentrations of 10 μc./ml. or less. The continued presence of chromatid aberration types results from the fact that the developing microspores are exposed to radiations from absorbed radiophosphorus during the entire period of their development, including their passage through prophase up to the metaphase stage at which observations can be made. Chromosome aberration types

appeared after the chromatid types, but were not observed before the fourth day, which is considerably later than was anticipated on the basis of the previous x-ray experiments. This delay in the appearance of break types produced at the resting stage may result from either (*a*) a retardation in the mitotic cycle in the microspores when subjected to continuous β-radiation from absorbed radiophosphorus, or (*b*) too low an initial radiation intensity to produce a detectable yield of these types—which in x-ray experiments have been shown to result principally from two breaks produced by independent, but temporally related electron paths.[2] Observations at radiophosphorus concentrations intermediate between those used in series A and B as well as tests of β-radiation from an intense external source should help distinguish between these possibilities.

TABLE 1

FREQUENCIES OF VARIOUS STRUCTURAL CHANGES PRODUCED IN CHROMOSOMES OF *Tradescantia* MICROSPORE NUCLEI FOLLOWING UPTAKE OF RADIOPHOSPHORUS BY INFLORESCENCES

TIME AFTER START OF EXPERIMENT	NO. OF CELLS EXAMINED	CHROMATID ABERRATIONS PER 100 CELLS: CHROMATID AND ISO-CHROMATID BREAKS	CHROMATID ABERRATIONS PER 100 CELLS: EXCHANGES	CHROMOSOME ABERRATIONS PER 100 CELLS: CHROMOSOME BREAKS	CHROMOSOME ABERRATIONS PER 100 CELLS: INTERSTITIAL DELETIONS	CHROMOSOME ABERRATIONS PER 100 CELLS: EXCHANGES (CENTRIC RINGS AND DICENTRICS)
Series B—Initial Concentration of P 32: 100 μc./Ml.						
24 hrs.	165	1.2	0.0	0.0	0.0	0.0
48 hrs.	127	13.4	2.4	0.0	0.0	0.0
74 hrs.	130	15.4	4.6	0.0	0.0	0.0
4 days	109	33.0	4.6	2.8	0.0	1.0
5 days	114	28.0	5.3	1.8	0.0	1.0
6 days	108	32.4	6.5	0.0	0.0	1.0
8 days	86	39.5	12.8	1.2	4.7	3.5
9 days	84	37.0	10.8	0.0	1.2	3.6
Series C—Initial Concentration of P 32: 1 μc./Ml.						
74 hrs.	146	2.7	0.0	0.0	0.0	0.0
4 days	136	5.9	0.7	0.0	0.0	0.0
9 days	116	11.2	1.7	0.0	1.7	1.7
Series D—Initial Concentration of P 32: 0.1 μc./Ml.						
4 days	142	2.8	0.0	0.0	0.0	0.0
9 days	134	2.3	0.0	0.0	0.0	0.0

Table 1 indicates that there is an increase in the frequency of aberrations with time. For series B this increase is approximately linear for chromatid and isochromatid breaks up to four days and is directly correlated with the regular increase in radioactivity of inflorescences as measured with the electroscope. Such a direct relationship is to be expected if these aberrations behave largely as one-hit types with β-radiation as they do with both x-rays and neutrons. Measurements are not yet available to determine

whether the apparent leveling-off in aberration frequency after about four days can be correlated with a corresponding behavior of the curve for radioactivity increase. In a few instances where direct measurements were made of the radioactivity of individual buds or anthers at a given time with a Geiger-Müller counter and the aberration frequency in the same material immediately studied, fairly good correlations of the two measurements were obtained. No systematic attempt has yet been made to determine what fraction of the aberrations is due exclusively to radiation originating within the cells of a given anther or bud, as compared with the irradiation of one bud or inflorescence by β-particles from another.

One striking result of the comparison of aberration frequency types was the relatively high yield of chromatid exchange break types as compared with simple break types. It was early demonstrated in x-ray experiments[3] that exchanges, in contradistinction to simple break types, are aberrations caused by two independent hits (electron paths) and show a time-intensity relationship, such that the yield for a given dose depends on the intensity—decreasing greatly at low intensities. Thus it might be expected that a relatively low yield of exchanges would be obtained with the prolonged radiation at low intensities from radiophosphorus. The relatively high frequency actually observed with β-radiation agrees with the recent analysis of Catcheside, Lea and Thoday[6] which suggests that an appreciable fraction of the exchanges produced at low intensities of x-rays are one-hit types, and further that the time of restitution of broken ends may not be as short as was originally thought.

At increasingly low initial external concentrations of radiophosphorus, decreases in aberration frequency result, though the yields of one-hit types are only roughly proportional to the original external radiophosphorus concentrations, indicating that differences in the degree of phosphorus uptake are probably involved. Even at quite low concentrations (series D), however, where the measured radioactivity of individual buds is of the same order of magnitude as that used in tracer experiments, chromosomal structural changes are still found. Thus it should be realized by investigators utilizing radioisotopes as tracers, especially with biological materials which are maintained in living condition after treatment and used as breeding stocks, that such treatment may result in the production of appreciable numbers of genetic changes. At higher levels of initial radiophosphorus concentration the aberration frequencies, even after relatively short times, may be equivalent to those produced by considerable doses of x-rays. In series A, after only 24 hours, the frequency of chromatid and isochromatid effects (35 per 100 cells) is equivalent to an exposure of the cells to approximately 40 roentgens;[6] in series B, after 9 days, this frequency is the same as that produced by about 50 r. Even though β-radiations are of restricted penetrating power as compared with certain

other radiations, these observations, plus the results of electroscope measurements of inflorescences permitted to take up radiophosphorus from relatively concentrated solutions, indicate that investigators should exercise considerable caution in the use of radioisotopes, especially as regards their close and prolonged exposure to biological materials which have absorbed appreciable quantities of these substances.

Summary.—Inflorescences of *Tradescantia paludosa* were permitted to take up radiophosphorus from solutions of various concentrations over a period of nine days. Cytological observations were made at the first post-meiotic (microsopore) mitosis at intervals following the initiation of the treatments to determine the types and frequencies of chromosome structural changes which might result from the β-radiation emitted by the radiophosphorus inside the plants. Changes were detected with all of the four concentrations of radiophosphorus used—between the limits of 100 μc./ml. and 0.1 μc./ml. Because of the very high breakage rate at the 100 μc./ml. concentration, aberration frequencies could not be scored after about 48 hours in this series.

The types of aberrations observed were similar to those resulting from the exposure of inflorescences to external radiations such as x-rays and neutrons. There was a fairly regular increase in the frequency of one-hit aberration types with time, which could be correlated with the increase in radioactivity of inflorescences as measured with an electroscope. Chromatid aberrations were observed within 24 hours after the initiation of the treatments and remained the principal type throughout the course of the experiments. Chromatid exchanges occurred with a relatively high frequency as compared with chromatid and isochromatid breaks even at the low radiation intensities involved. Chromosome breaks were not observed until the fourth day. At the highest level of radiophosphorus used (100 μc./ml.) the frequency of one-hit chromatid aberration types after 24 hours, under the experimental conditions utilized, was equivalent to that produced by about 40 r of x-rays.

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INTERPOLATION AND UNIQUENESS OF ENTIRE FUNCTIONS

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Let $\{T_n\}$ be a sequence of linear functionals defined on the class K of entire functions of exponential type. We raise the following questions:

(I) Given a sequence of complex numbers $\{a_n\}$ and a subclass C of K, is it possible to find a function f belonging to C such that for all n, $T_n(f) = a_n$?

(II) Given a subclass C of K and a function f belonging to C, what properties of f can be inferred from the sequence of complex numbers $\{T_n(f)\}$?

(III) For what subclass C of K is it true that if f belongs to C and $T_n(f) = 0$ for all n, then $f(z) \equiv 0$?

The third is of course a special case of the second, but it is of such special importance that we choose to state it explicitly. A class C for which it holds will be called a uniqueness class for $\{T_n\}$. The first question asks for a solution to the general interpolation problem for the class C and the functionals $\{T_n\}$; if a function exists having these properties, the sequence $\{a_n\}$ is said to be admissible for the functionals $\{T_n\}$ and the class C. The importance of (III) stems from the fact that if, in (I), C is a subclass of such a uniqueness class then there can exist at most one such function f for which $T_n(f) = a_n$, while if, in (II), C is a subclass of a uniqueness class, f is in fact completely determined by the sequence $\{T_n(f)\}$ and every property of f should be inferable from it. This leads naturally to an additional question.

(IV) When is it possible to expand $f(z)$ into a series of the form

$$f(z) = \sum u_n(z) T_n(f)?$$

This is the problem of the existence of an interpolation series for the functionals $\{T_n\}$, together with the determination of the class of functions for which the series converges. Clearly, any such expansion class is a subclass of the corresponding uniqueness class. In a previous paper,[1] certain aspects of these problems were discussed. Here, the methods used there—due essentially to Pólya[2] and Carlson[3]—are given in a more general form, and are applied to the solution of problem (IV). The treatment is similar to that of Gelfond,[4] but is of much greater power; most of the known results on convergence of Abel, Newton, Stirling, Selberg, as well as other less familiar interpolation series, are obtained at once, and the method extends to discussion of summability. In the present paper, the general method is outlined briefly, and a few of the specific results are stated; detailed proofs will appear in a later paper.

We first seek general representations for functions f and functionals T. Some preliminary definitions are necessary. If $f \in K$, the class of entire functions of exponential type, so that there exist real numbers A and C such that $|f(z)| \leqslant Ae^{C|z|}$, then the growth function $h(\theta, f)$ defined by $\limsup_{r\to\infty} r^{-1} \log |f(re^{i\theta})|$ is bounded in absolute value by C. If $H(\theta)$ is any function with period 2π, then $K(H(\theta))$ denotes the class of all f in K such that $h(\theta, f) \leqslant H(\theta)$ for all θ; $H(\theta)$ may take infinite values. As in previous papers, the notation $K(a, c)$ is reserved for the class of all f in K with $h(0, f) \leqslant a$, $h(\pi, f) \leqslant a$, and $h(\pm \frac{\pi}{2}, f) \leqslant c$. If G is any closed set of the complex plane, $k(\theta, G) = \sup_{w \in G} \Re\{we^{i\theta}\}$ is called the supporting function of G; this differs from the usual definition in the sign of θ. The function $k(\theta, G)$ is continuous in θ, has period 2π, has left and right derivatives everywhere, and is unchanged if G is replaced by its convex hull. If G_1 and G_2 are point sets, then $G_1 \cdot G_2$ will be the set of all points of the form $z'z''$ where $z' \in G_1$, $z'' \in G_2$.[5] If G_1 and G_2 are bounded so is $G_1 \cdot G_2$; if G_1 and G_2 are closed and compact, so is $G_1 \cdot G_2$; if G_1 and G_2 are closed bounded convex sets, then $G_1 \cdot G_2$ is simply connected; $G_1 \cdot G_2$ is a star set whenever one factor is a star set. If $f(z) = \sum_0^\infty a_n z^n/n!$, belonging to K, then $\phi(w) = \sum_0^\infty a_n/w^{n+1}$ is usually called the Borel transform of f. We denote by $D(f)$ the convex hull of the singularities of $\phi(w)$. In our notation, a fundamental theorem due to Pólya[2] states that if f belongs to K then $h(\theta, f) = k(\theta, D(f))$ for all θ.

Suppose we consider a function $\gamma(z) = \sum_0^\infty z^n/n!\, c_n$ and an associated function $\gamma^*(z) = \sum_0^\infty c_n z^n/n!$, both in K. A modified Borel transform of $f(z)$ is defined by $\phi(w) = \sum_0^\infty a_n c_n/w^{n+1}$. $\phi(w)$ is regular outside the set $D(f) \cdot D(\gamma^*)$, and if Γ is any contour enclosing this set, $f(z)$ has the representation

$$f(z) = \frac{1}{2\pi i} \int_\Gamma \gamma(zw)\phi(w)dw. \tag{1}$$

Conversely, if $\gamma \in K$, if G is a closed bounded simply connected set, if $\phi(w)$ is regular outside G and if Γ is a contour enclosing G and not passing through the origin, then $f(z)$, defined by (1), belongs to K, and $h(\theta, f) \leqslant k(\theta, G \cdot D(f))$. (The special case for which $\gamma(z) = e^z = \gamma^*(z)$ was studied by Pólya; it is of interest that if $D(\gamma) = D(\gamma^*) = 1$, then $\gamma(z) = Ae^z$.)

Turning now to functionals, we may obtain a wide variety as follows. Let $\phi(w)$ be a modified Borel transform of f with kernel $\gamma(z)$, and choose any contour Γ enclosing $D(f) \cdot D(\gamma^*)$. Then, for any entire function $g(w)$, we define T by:

$$T(f) = \frac{1}{2\pi i} \int_\Gamma g(w)\phi(w)dw. \tag{2}$$

This includes all of the usual cases. In fact, if K is given the weak topology, any continuous linear functional on K may be represented in the form (2). When T is so defined, $g(w)$ is said to be the generating function of T.

With this background, we may now study general interpolation series. For simplicity, we confine ourselves to the case where the functionals $\{T_n\}$ have generating functions $\{g_n(w)\}$ which are exactly the integral powers of a function $\zeta(w)$. This restriction is indeed satisfied for most of the familiar series. If $\zeta(w)$ is regular and univalent in an open set Ω_w of the w-plane, containing the origin, and if Ω_w corresponds to a set Ω_ζ in the ζ-plane, then $w = w(\zeta)$, regular in Ω_ζ. Since $\gamma(zw) = \gamma(zw(\zeta))$ is analytic in ζ for ζ in $\Omega\zeta$,

$$\gamma(zw) = \sum_0^\infty u_n(z)\zeta^n \tag{3}$$

uniformly convergent in any closed subset of Δ_ζ, the largest open circle $|\zeta| < R$ contained in Ω_ζ. Let Δ_w be the image of Δ_ζ, given by $|\zeta(w)| < R$. Then

$$\begin{aligned} \gamma(zw) &= \sum_0^\infty u_n(z)[\zeta(w)]^n \\ &= \sum_1^\infty u_n(z)g_n(w) \end{aligned} \tag{4}$$

is uniformly convergent in any closed subset of Δ_w. If f is such that $D(f) \cdot D(\gamma^*)$ is interior to Δ_w, then we can choose a contour Γ lying in Δ_w and enclosing $D(f) \cdot D(\gamma^*)$, on which (4) is uniformly convergent. Applying (1) and (2), and integrating termwise, we obtain the convergent interpolation series

$$f(z) = \sum_0^\infty u_n(z)T_n(f). \tag{5}$$

The same procedure will yield results for summability. Let $E(t) = \sum_0^\infty d_n t^n$ be an entire function, not a polynomial, with $d_n \geqslant 0$. If $\{S_n\}$ is a sequence of complex numbers with $|S_n|^{1/n} = 0(1)$ then $\sum_0^\infty S_n d_n t^n$ converges for all t to a function $H(t)$. The limit $\lim_{t \to +\infty} H(t)/E(t)$, if it exists, is the generalized E-limit of $\{S_n\}$. Applied to a power series, the following is true: if $f(z)$ is regular in a region R containing the origin, then

$$f(z) = E - \sum_0^\infty f^{(n)}(0)\, z^n/n! \tag{6}$$

where the summability is uniform in any closed compact subset of $(R' \cdot E')'$. Here, E is an open set such that $\lim_{t \to \infty} E(zt)/E(t)$ is zero, uniformly in any compact subset of E.

Returning now to our discussion of interpolation series, we take up E-summability. Since $\gamma(zw)$, as a function of ζ, is regular in Ω_ζ, we have

$$\gamma(zw) = E - \sum_0^\infty u_n(z)\zeta^n$$

uniformly in any compact subset of $\Omega_\zeta^* = (\Omega_\zeta' \cdot E')'$. If Ω^w is the image of Ω_ζ^* under $w = w(\zeta)$, then

$$\gamma(zw) = E - \sum_0^\infty u_n(z) g_n(w)$$

uniformly in any compact subset of Ω_w^*. Proceeding as before, we substitute this into (1), and integrate termwise; simplifying by (2), we have

$$f(z) = E - \sum_0^\infty u_n(z)\, T_n(f), \tag{7}$$

holding for all f such that $D(f) \cdot D(\gamma^*)$ lies interior to Ω_w^*. We summarize these results as follows.

THEOREM. *Let $g_n(w) = [\zeta(w)]^n$ be the generating functions of the functionals $\{T_n\}$. Then, the formal interpolation series $\sum_0^\infty u_n(z)T_n(f)$ converges to $f(z)$ for all f such that $D(f) \cdot D(\gamma^*)$ lies in Δ_w, and is E summable to $f(z)$ for all f such that $D(f) \cdot D(\gamma^*)$ lies in Ω_w^*.*

By specialization of the kernel $\gamma(z)$, and the function $\zeta(w)$ and the function $E(t)$ determining the method of summability, many specific results, both new and old, can be obtained. We indicate briefly only a few of these; more detailed treatments will appear later.

THEOREM 1. *The Newton expansion $\sum_0^\infty \binom{z}{n} \Delta^n f(0)$ is convergent to $f(z)$ if $h(\theta, f) < \theta \sin\theta + \cos\theta \log(2\cos\theta)$, is Borel summable to $f(z)$ if $f \in K(a, c)$ with $c < \frac{\pi}{2}$, and is Mittag-leffler summable to $f(z)$ if $f \in K(a, c)$, with $c < \pi$.*

COROLLARY (Carlson[6]). *If $f \in K(a, c)$, $c < \pi$, and $f(n) = 0$ for $n = 0, 1, 2, \ldots$, then $f(z) = 0$.*

THEOREM 2. *The Abel series $\sum_0^\infty z(z - n)^{n-1} f^{(n)}(n)/n!$ is convergent to $f(z)$ if $D(f)$ lies in the region $|we^{1+w}| < 1$, and is ML summable to $f(z)$ if*

$h(\theta, f) < k(\theta, \Omega_w^*)$ where Ω_w^* is the region bounded by the curve $\rho = (\pi - |\varphi|)/\sin|\varphi|$.

COROLLARY. *If $f \in K(A)$, $A < 1$, and $f^{(n)}(n) = 0$ for $n = 0, 1, \ldots$, then $f(z) = 0$.*

THEOREM 3. *The Stirling series $f(0) + \sum_1^\infty \frac{z}{n}\binom{z-1+n/2}{n-1}\Delta^n f\left(-\frac{n}{2}\right)$ is convergent to $f(z)$ if $D(f)$ lies in the region $|\sinh(w/2)| < 1$, is Borel summable to $f(z)$ if $f \in K(\pi/\sqrt{2})$, and is ML summable if $f \in K(a, c)$ with $c < \pi$.*

THEOREM 4. *The series $f(0) + \sum_1^\infty \frac{z}{n}\binom{z-n-1}{n-1}\Delta^n f(n)$ is convergent to $f(z)$ if $f \in K(A)$, with $A < \log(1+\sqrt{2})/2$ and is ML summable if $h(\theta, f) < (-\theta)\sin\theta + \cos\theta \log(-2\cos\theta)$ for $\frac{\pi}{2} \leqslant |\theta| \leqslant \pi$.*

THEOREM 5. *If $D(f)$ lies in the region bounded by the curve $u = \log\sin\beta v - \log\sin(\beta+1)v$, for $\beta > 0$, then*

$$f(z) = ML - \left\{f(0) + \sum_1^\infty \frac{z}{n}\binom{z-\beta n-1}{n-1}\Delta^n f(\beta n)\right\}.$$

Several of the above theorems can be strengthened considerably, but for simplicity of statement only the weaker forms have been given. In particular, the uniqueness theorems obtained by Gelfond[4] by methods of conformal mapping become immediate corollaries of theorems on the summability of the corresponding interpolation series. It is also possible to treat series arising from sequences of functionals $\{T_n\}$ whose generating functions are not of the form $[\xi(w)]^n$. The Lidstone series[7] is a simple example of such a series.

THEOREM 6. *The Lidstone series $\sum_0^\infty \Lambda_n(z) f^{(2n)}(1) + \sum_0^\infty \Lambda_n(1-z) f^{(2n)}(0)$ are convergent to $f(z)$ if $f \in K(A)$, $A < \pi$, are Borel summable to $f(z)$ if $f \in K(a, c)$, $c < \pi$, and are ML summable to $f(z)$ if $D(f)$ does not contain any points of the vertical lines $u = 0$, $|v| \leqslant \pi$.*

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ON A THEOREM OF BANACH

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Consider a trigonometric series

$$\sum_{n=1}^{\infty}(a_n \cos nx + b_n \sin nx) \tag{1}$$

and any sequence of positive integers $n_1 < n_2 < \ldots$ satisfying an inequality

$$n_{k+1}/n_k > q > 1 \qquad (k = 1, 2, \ldots)$$

where q is independent of k. Such sequences $\{n_k\}$ are often called *lacunary*. The following two results are due to Banach:[1]

(i) *Given any sequence* $\alpha_1, \beta_1, \alpha_2, \beta_2, \ldots$ *of real numbers tending to 0, there is always a Fourier-Lebesgue series* (1) *such that*

$$a_{n_k} = \alpha_k, \quad b_{n_k} = \beta_k \qquad \text{for } k = 1, 2, \ldots \tag{2}$$

(ii) *Given any sequence of real numbers* $\alpha_1, \beta_1, \alpha_2, \beta_2, \ldots$ *such that* $\Sigma(\alpha_k^2 + \beta_k^2) < \infty$, *there is always a series* (1) *which is the Fourier series of a continuous function* $f(x)$ *and which satisfies* (2).

While there exist simple proofs of (i), based on the considering of the (F. Riesz') products[2]

$$\prod_{k=1}^{\nu}(1 + \alpha_k \cos n_k x + \beta_k \sin n_k x) \tag{3}$$

the existing proofs of (ii) are long and complicated. In this note we shall show that a simple modificition of the product (3) immediately leads to a proof of (ii).

First of all, we observe that it is enough to prove a slightly weaker variant of (ii), with the condition of continuity of f there replaced by that of boundedness. For let $\epsilon_1, \epsilon_2, \ldots$ be any positive and convex sequence of numbers tending to 0, so slowly, however, that $\sum(\alpha_k^2 + \beta_k^2)\epsilon_{n_k}^{-2}$ still converges. Suppose that (ii) has been established in the weaker form just mentioned. Then there is a series (1) which is the Fourier series of a bounded function f and such that

$$a_{n_k} = \alpha_k \epsilon_{n_k}^{-1}, \qquad b_{n_k} = \beta_k \epsilon_{n_k}^{-1}. \tag{4}$$

It is known however[3] that if we multiply the nth term of the Fourier series

of a bounded function by ϵ_n $(n = 1, 2, \ldots)$ the resulting series is the Fourier series of a continuous function. Thus $\sum(a_n \cos nx + b_n \sin nx)\epsilon_n$ is the Fourier series of a continuous function, and, by (4), the coefficients of the latter series at the places n_k are α_k, β_k.

Passing to the proof of (ii), with f merely bounded, let us consider the products

$$P_\nu = i^{-1} \prod_{k=1}^{\nu} \{1 + iA_k(x)\} \qquad (\nu = 1, 2, \ldots) \tag{5}$$

where $i = \sqrt{-1}$ and $A_k(x) = \alpha_k \cos n_k x + \beta_k \sin n_k x$. These products are very similar to (3), so that certain well-known properties of the latter[4] remain obviously valid. Let us list them:

(*a*) For $q \geqslant 3$, if we multiply P_ν out and replace the products of cosines and sines by linear combinations of cosines and sines, all the terms we get are distinct. In particular, the terms of P_ν with indices n_k $(k = 1, 2, \ldots, \nu)$ are $\alpha_k \cos n_k x + \beta_k \sin n_k x$.

(*b*) For $q \geqslant 3$, P_ν is a partial sum of $P_{\nu+1}$.

Hence assuming that $q \geqslant 3$ and making $\nu \to \infty$ in (5) we obtain formally a trigonometric series (1) satisfying (2). The $P_\nu(x)$ are uniformly bounded, since

$$|P_\nu| \leq \{\prod_{k=1}^{\nu}(1 + \alpha_k^2 + \beta_k^2)\}^{1/2} \leq \{\prod_{k=1}^{\infty}(1 + \alpha_k^2 + \beta_k^2)\}^{1/2} < \infty.$$

It immediately follows that the so-obtained series is the Fourier series of a bounded function $f(x)$, and (ii) is proved for $q \geqslant 3$. Our function f is in general complex valued, but obviously the real part of $f(x)$ also satisfies the required conditions.

To get rid of the condition $q \geqslant 3$ we proceed as in the proof of (i). As there,[5] we show that

(*c*) Let ϵ be a fixed but arbitrarily small positive number. If q is large enough, $q \geqslant q_0(\epsilon)$, all the indices μ actually occurring in the trigonometric polynomial P_ν—and so also of the series (1) which is the formal limit of the P_ν—are situated in the intervals $(n_k(1 - \epsilon), n_k(1 + \epsilon))$.

Let now q be any number greater than 1. Let r be any positive integer, and let us split the sequence $\{n_k\}$ into r sequences

$$n_k^{(s)} = n_{s+kr} \qquad (s = 1, 2, \ldots, r;\ k = 0, 1, 2 \ldots)$$

Obviously $n_{k+1}^{(s)}/n_k^{(s)} > q^r$ can be as large as we please, provided r is large enough. In particular, we require that $q^r \geqslant 3$. We can construct a series (1), which we shall denote by T_s $(s = 1, 2, \ldots, r)$, such that T_s is the Fourier series of a bounded function and that T_s has the prescribed coefficients $(= \alpha_{s+kr}, \beta_{s+kr})$ at the places n_{s+kr}. In addition, if r is large enough, T_s will only have indices in the intervals $(n_{s+kr}(1 - \epsilon), n_{s+kr}$

$(1 + \epsilon)$). Hence, if r is large enough, the series $T_1, T_2, \ldots, T_r$ do not overlap, and the series $T = T_1 + T_2 + \ldots + T_r$ has the required properties.

[1] For the literature, see Zygmund, A., *Trigonometrical Series* (quoted hereafter *TS*), p. 215.

[2] See *TS*, p. 220.

[3] *TS*, pp. 104, 105.

[4] *TS*, p. 139.

[5] *TS*, p. 139.

THE INVERSION OF A GENERALIZED LAPLACE TRANSFORM

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In these PROCEEDINGS[1] the author showed how the convolution transform

$$f(x) = \int_{-\infty}^{\infty} G(x - t)\phi(t)dt \tag{1}$$

can be inverted by a linear differential operator of infinite order. The kernel $G(x)$ was any function of the form

$$G(x) = \frac{1}{2\pi i}\int_{c-i\infty}^{c+i\infty} \frac{e^{st}}{E(s)}ds \qquad 0 < c < a_1, \tag{2}$$

where

$$E(s) = s\prod_{k=1}^{\infty}\left(1 - \frac{s^2}{a_k^2}\right)$$

$$0 < a_1 < a_2 < \ldots$$

$$\sum_{k=1}^{\infty}\frac{1}{a_k^2} < \infty.$$

The inversion of the Stieltjes transform was included as the special case $a_k = k$, $E(s) = \pi^{-1}\sin \pi s$. The Laplace transform was not included in the general theory since, as we shall see, it requires the introduction of a function $E(s)$ whose roots are not symmetrically situated with respect to the origin.

Let us make an exponential change of variable in the Laplace transform

$$F(s) = \int_0^{\infty} e^{-st}\Phi(t)dt. \tag{3}$$

Then equation (3) takes the form (1) with

$$f(x) = F(e^{-x}), \quad G(x) = e^{-e^{-x}}, \quad \phi(x) = \Phi(e^x)e^x.$$

Since

$$\Gamma(s) = \int_0^\infty e^{-u}u^{s-1}du = \int_{-\infty}^{\infty} e^{-st}e^{-e^{-t}}dt,$$

we have by the classical inversion of the bilateral Laplace transform that

$$e^{-e^{-x}} = \frac{1}{2\pi i}\int_{c-i\infty}^{c+i\infty} e^{sx}\Gamma(s)ds \qquad 0 < c < \infty.$$

This equation has the form (2) with $E(s)$ replaced by $1/\Gamma(s)$, an entire function with its roots at the points $0, -1, -2, \ldots$. With this example before us, let us now proceed with the more general theory.

Set

$$E(s) = s^p \prod_{k=1}^{\infty}\left(1 - \frac{s}{a_k}\right)e^{s/a_k}, \tag{4}$$

where p is a non-negative integer and the a_k are real constants such that

$$0 < |a_1| \leqq |a_2| \leqq \ldots \tag{5}$$

$$\sum_{k=1}^{\infty}\frac{1}{a_k^2} < \infty. \tag{6}$$

Under these conditions it can be proved that $1/E(s)$ is a bilateral transform of a function $G(x)$ which we take to be the kernel of the transform (1). Our principal result is that this transform can be inverted by the linear differential operator of infinite order $E(D)$. That is,

$$E(D)f(x) = \phi(x)$$

$$= \lim_{n\to\infty} D^p \prod_{k=1}^{n}\left(1 - \frac{D}{a_k}\right)e^{D/a_k} f(x)$$

$$= \lim_{n\to\infty} D^p \prod_{k=1}^{n}\left(1 - \frac{D}{a_k}\right) f(x + s_n),$$

where D stands for differentiation with respect to x and

$$s_n = \sum_{k=1}^{n}\frac{1}{a_k}.$$

We state the result in the form of a theorem.

THEOREM. *If $\phi(x)$ is continuous and absolutely integrable on $(-\infty, \infty)$, if $E(s)$ is defined by equations (4) (5) (6), $G(x)$ by (2) and $f(x)$ by (1), then*

$$E(D)f(x) = \phi(x). \tag{7}$$

Let us now show that the Post-Widder[2] inversion formula

$$\lim_{n\to\infty} \frac{(-1)^n}{n!}\left(\frac{n}{t}\right)^{n+1} f^{(n)}\left(\frac{n}{t}\right) = \Phi(t) \tag{8}$$

for the Laplace transform (3) is included in the above theorem. Let us use the Euler[3] form of the infinite product expansion of $\Gamma(s)$:

$$\frac{1}{\Gamma(s)} = s\prod_{k=1}^{\infty}\left(1+\frac{s}{k}\right)\left(1+\frac{1}{k}\right)^{-s}$$

$$= \lim_{n\to\infty} n^{-s}\, s\prod_{k=1}^{n}\left(1+\frac{s}{k}\right).$$

Then equation (7) becomes

$$\lim_{n\to\infty} D\prod_{k=1}^{n}\left(1+\frac{D}{k}\right) f(x-\log n). \tag{9}$$

But if we set $e^{-x} = t$ and observe the relation $D_x = -tD_t$, equation (9) becomes

$$\lim_{n\to\infty} -tD_t\prod_{k=1}^{n}\left(1-\frac{tD_t}{k}\right)F(nt) = \Phi\left(\frac{1}{t}\right)\frac{1}{t}.$$

Simple computation shows that this equation is equivalent to

$$\lim_{n\to\infty} \frac{(-nt)^{n+1}}{n!} F^{(n+1)}(nt) = \Phi\left(\frac{1}{t}\right)\frac{1}{t}.$$

If t is replaced by its reciprocal this equation is seen to be the same as (8).

It is important to note the equality signs in the relations (5). Thus multiple roots are permitted in the function $E(s)$. As a consequence all the successive iterated Laplace transforms are subsumed under the present theory. They are inverted by use of equation (7) where $E(s)$ has multiple roots (in the case of iterates beyond the first). That is, suitable modifications of the Post-Widder operator will be effective.

[1] Widder, D. V., "Green's Functions for Linear Differential Systems of Infinite Order," *Proc. Nat. Acad. Sci.*, **33**, 31–34 (1947).

[2] Widder, D. V., "The Laplace Transform," Princeton (1946), p. 288.

[3] Whittaker, E. T., and Watson, G. N., "A Course of Modern Analysis," Cambridge (1943), p. 237.

THE IODINE METABOLISM OF DROSOPHILA GIBBEROSA STUDIED BY MEANS OF RADIOIODINE I^{131}*

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Introductory.—The very little knowledge available concerning the iodine metabolism of invertebrates is confined to the occurrence of accumulation of the element in sponges and gorgonians.[1] It therefore seemed desirable to attack the problem by using I^{131} in some other organism. Because *Drosophila gibberosa* is a holometabolous insect, it seemed that valuable information might be obtained which would not only be of interest from the standpoint of iodine metabolism of this fly but would also provide a convenient basis for comparative work on the iodine metabolism of some other insect or arthropod.

D. gibberosa, a giant black Mexican species, was sent to this laboratory through the courtesy of Dr. T. Dobzhansky. The size of *D. gibberosa* as compared with the smaller *D. melanogaster* is of considerable advantage especially in carrying out the technical side of this problem.

Material and Methods.—Radioactive carrier-free iodide was made available to both third instar larvae and adults by stirring 0.2 ml. of a solution of the isotope into approximately 8 ml. of liquid corn meal, molasses and dried Brewer's yeast mixture which had been placed in a creamer bottle. The largest quantities of I^{131} used were 0.2 mc.

TABLE 1

Jaws and Anterior Fore Gut Chitin	34.3%
Larval Skin	29.8
Tracheal Trunks and Posterior Spiracles	20.1
Imaginal Discs	3.1
Fat Body	2.7
Ring Gland	3.6
Fore and Mid Gut	0.0
Hind Gut	2.7
Salivary Glands	0.0
Malpighian Tubes	2.7
Cerebral Ganglia and Nerve Cord	0.94

Both larvae and adults were allowed to feed on the radioactive food for varying periods of time, after which dissections were made and measurements of activity taken with a Geiger counter. A second more sensitive method for detecting radioactivity was employed with the larvae. This involved making radioautographs by mounting alternate paraffin ribbon sections of the larvae on dental x-ray film, exposing for periods of 3–5 days

and developing after removal of the paraffin. The other alternate series was mounted on glass slides for iron hematoxylin staining.

Larval Distribution.—Table 1 indicates the distribution of I^{131} in a third instar larva after feeding on radioactive medium for 72 hours. The percentages in this instance were calculated on the basis of the sum of the individual Geiger counts from each dissection in this experiment, disregarding the loss of activity due to dissection of the larva in either alcohol or insect Ringer. No counts are available in this instance to indicate the amount of activity left in the dissection fluid, but as the tissues were dissected in great detail, the data form a convenient introduction to further discussion.

These results indicate clearly that the iodine is concentrated in the skeletal parts of the larva. It is worth noting that no concentration of I^{131} appeared in any of the larval endocrine structures.

Series of experiments were carried out in which third instar larvae fed on the radioactive food for six different time intervals varying from $17^1/_2$–96 hours. After removal from the bottles, and following a washing in insect Ringer solution in order to remove any excess food adhering to the surface, Geiger counts were taken on whole larvae which had been teased apart. These larvae were then removed from the Geiger counter, dissected completely and counts taken on the individual tissues dissected. The mean per cent of activity recovered from eleven such series of counts on dissected larvae was 125.2% of that of counts on the teased larvae. The variation in excess of 100% is undoubtedly due to two factors. In the first place, it is impossible to obtain completely accurate counts on the teased entire larvae due to the thickness of the incompletely separated tissues which must absorb some β-radiation. In the second place, it is extremely difficult to duplicate precisely the identical geometrical counter relationships for each individual dissection made.

TABLE 2

MEAN PERCENTAGES OF RADIOACTIVITY

Larval Skin	72.7%
Jaws, Anterior and Posterior Spiracles	12.7
Tracheal Trunks	2.8
Residue	11.6

The teased larvae were separated into the following dissected parts: larval skin; tracheal trunks; jaws, anterior and posterior spiracles; and residue which included all remaining tissues and body fluids of the organism. Table 2 indicates the mean of the percentages of activity found in these parts. The sum of the counts for the four individual dissections was used as 100% in calculating the percentages. The age of the larva apparently is not a factor in determining the percentage of radioactivity in the larval skin.

The radioautographs likewise show clearly the concentration of radioactivity which is present in the skeletal structures of the larva. Figure 1 is a longitudinal section, not including the extreme anterior end, cut across the dorsal surface of a third instar larva. A radioautograph, figure 2, made from an alternate section of the same third instar larva as seen in figure 1, indicates that the concentration of greatest radioactivity was in the larval skin and tracheal trunks. The cross section of a third instar larva seen in figure 3 is cut through the hind gut and anal regions. Attention should be directed to the very large hypodermal cells located ventrally and extending only to the midlateral regions of the larva. Figure 4 shows that the radioactivity was extremely high in this region. It seems likely that these large cells have in some way been active in concentrating the radioiodine in the cuticle under which they lie.

Skins of the larvae which were highly radioactive were treated for 48 hours with hot 5% KOH (65°C.). At the end of this period, Geiger counts indicated that virtually all of the radioactive material had been removed, none being left in the chitin fractions which remained after the KOH treatment. This suggests that the radioiodine is present in a protein portion of the skin rather than in the chitin.[2, 3]

Inasmuch as the larvae were crawling about in the radioactive food, the question arises as to whether the iodine in the exoskeleton may represent purely physical uptake by the external surface of the animal. When fresh-cleaned skins of third instar larvae were placed in a radioactive iodide solution for a period as long as six days, there was no indication from Geiger counts that any I^{131} had been removed from the medium. If physical uptake were responsible for the radioactivity found in the intact larval skins, the skins immersed in iodide solution would presumably have given some

Explanation of Figures

Abbreviations: A, Anus; FB, Fat Body; HG Hind Gut; LHC, Large Hypodermal Cells; LS, Larval Skin (includes both hypodermal cells and underlying cuticle); M, Muscle; TT, Tracheal Trunk.

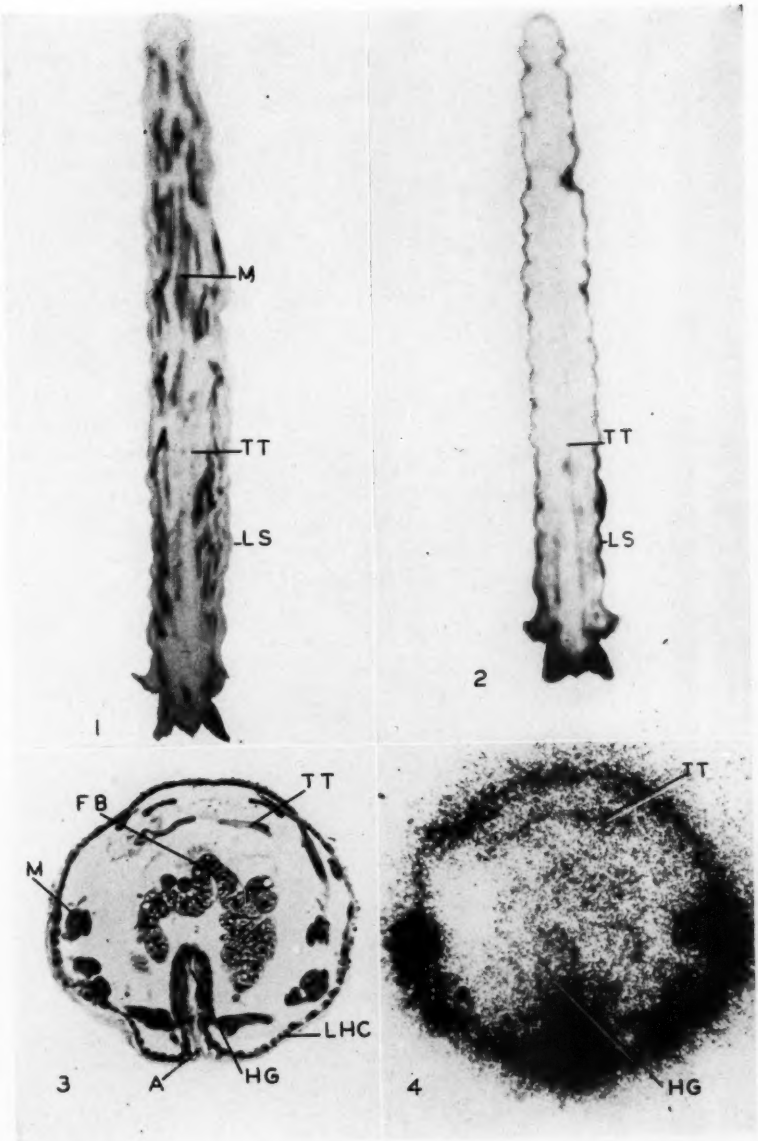
Longitudinal sections, × 23; cross sections, ×96.

Figure 1. Longitudinal section of a third instar larva stained with iron hematoxylin.

Figure 2. Radioautograph made from the longitudinal section adjacent to that in figure 1. The darkened areas represent reduced regions of the x-ray film which coincide with the accumulation of I^{131} in the tissues sectioned.

Figure 3. Transverse section through the anus and hind gut region of a third instar larva stained with iron hematoxylin. Note the general topography for interpretation of figure 4.

Figure 4. Radioautograph made from the cross section adjacent to that in figure 3. The marked I^{131} accumulation located ventrally and extending midlaterally in the section coincides with the region of the large hypodermal cells and adjacent larval cuticle figure 3.



(For explanation of figures see opposite page).

indication of these processes. If second instar larvae which have been feeding on I^{131} are removed from the food just prior to molting, and are washed and then allowed to molt after crawling on moist filter paper, Geiger counts of the young third instar larvae indicate a high degree of radioactivity. There is no possible way for these larvae to have acquired this amount of radioactivity except by first feeding and then depositing the I^{131} in the skeletal components. It is therefore legitimate to conclude that the deposition of iodine in the protein of the skeleton represents a genuine metabolic treatment by the organism of iodine taken up in the food.

Pupal Period.—Larvae which had fed on radioactive food were allowed to pupate in the bottles. The pupae (ages 24 hours and 96 hours) were then removed, washed and dissected free of the pupa cases. Geiger counts were taken on samples, each of which consisted of a tanned puparium and its pupa. These were then separated completely and individual counts made on the puparium and pupa. The mean of the percentage of activity recovered from the completely separated portions was 114.3%. The reasons for this mean being in excess of 100% have already been stated in the case of the larvae. When the sum of the counts for the two individual dissected structures was used as 100%, the mean percentage of radioactivity in the puparium was 85.0%, in the pupa, 14.9%. Inasmuch as the pupal skin remained intact, it seems likely that most of the 14.9% of activity recorded for the pupa was located in the pupal skin, which is very hard to separate, rather than in the developing imago or the histolysed larval tissues. In contrast to the larval skin, the tanned puparium can incorporate I^{131} if placed in an iodide solution.

Larval Acquisition.—Adult Emergence. Pupae which had formed from larvae having fed on I^{131} were removed from the food before emergence of the imagos, washed and placed on moist filter paper in a vial. After emergence of the adults, complete dissections were made and counts taken on the dissected tissues. In no instance was there any significant radioactivity. It would appear that the larva and subsequently the pupa use the molted exoskeletons as a depot for any iodine that may enter. As far as the pupa and imago are concerned, the element can have little if any physiological significance. It should be noted that the quantity of iodine actually administered in a carrier-free I^{131} solution is excessively small and therefore likely to give a true picture of the movement of minute natural concentrations of stable iodine isotopes in the food.

Adult Acquisition.—Pupae, formed from larvae which had never fed on I^{131}, were buried in the radioactive food and imagos allowed to emerge, thereby assuring the adults of radioactive food only. When counts were taken on dissections of tissues of the adults after 4–5 days' feeding, the highest percentages of radioactivity were located again in the exoskeleton.

Table 3 includes all dissections which indicated 10% or more of the total activity calculated on the basis of the sum of the individual counts being equal to 100%.

TABLE 3

Thoracic Exoskeleton	31.6%
Legs	18.1
Abdominal Exoskeleton	14.0
Head (—the Eyes)	10.3

Additional data indicate that the iodine is probably in association with a protein complex in the adult exoskeleton as is the case in the larva. When portions of radioactive adult exoskeletons were treated with hot 5% KOH, the activity was lost. The chitin which remained after the treatment was free of any radioactivity.

Geochemical and Comparative Biochemical Significance.—It seems probable that the capacity to link iodine to a protein complex in the exoskeleton is characteristic not only of *D. gibberosa* but is typical of other insects and possibly other arthropods as well. If one considers the large amount of oceanic crustacean plankton, and if the organisms comprising this population also can form iodinated scleroproteins, an extremely large amount of the iodine present in the ocean might well be bound by their exoskeletons and be continually sedimented as exuviae.

It is known that some sponges and gorgonians accumulate large amounts of bromine and iodine in the form of dibromotyrosine and diiodotyrosine.[1] In view of the results obtained on *D. gibberosa*, it is reasonable to suppose that the concentration of these two halogens in the skeletons of such organisms is an inevitable result of the formation of scleroproteins in sea water containing iodine and bromine. If tyrosine is an important component amino acid of the protein complex in the larval skin of *D. gibberosa*, it should be possible to demonstrate traces of diiodotyrosine in the exoskeleton of this insect as well as in the lower invertebrates.

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